

Table S1. Statistical analysis.

Figure	Condition 1	N1	Median (min)	Average (min)	Condition 2	N2	Median (min)	Average (min)	MW-Wilcoxon Statistical test p value
4E_ProTAME (12μM)-induced delay in mitotic entry correlates with the time of addition after release from thymidine block	DMSO	150	612	625.0	proTAME at 0hr	150	696	712.8	0
					proTAME at 2hrs	150	660	669.0	3.97E-11
					proTAME at 4hrs	150	624	636.6	0.07
					proTAME at 6hrs	150	612	619.4	0.88
					proTAME at 8hrs	150	612	622.6	0.96
5A_ProTAME induced mitotic arrest in HeLa cells	DMSO	150	96	121.4	proTAME 780nM	150	156	165.4	1.43E-12
					proTAME 3μM	150	348	444.6	0
					proTAME 12μM	150	1680	1640.5	0
					proAAME 12μM	150	84	100.5	0.42
5B_Partial Cdc20 knockdown and low proTAME concentration synergize in delaying mitosis in HeLa cells	Control siRNA DMSO	153	60	72.8	Cdc20 siRNA DMSO	150	96	144.2	0
					Control siRNA proTAME	114	288	496.5	0
	Cdc20 siRNA DMSO	150	96	144.2	Cdc20 siRNA proTAME	112	1170	1235.3	0

Figure	Condition 1	N1	Median (fraction remaining)	Average (fraction remaining)	Condition 2	N2	Median (fraction remaining)	Average (fraction remaining)	MW-Wilcoxon Statistical test p value
5D_ProTAME stabilizes CyclinB1	proAAME	31	0.15	0.15	Nocodazole	34	0.83	0.87	3.87E-08
					proTAME	30	1.17	1.17	2.07E-11
	Nocodazole	34	0.83	0.87	proTAME	30	1.17	1.17	7.86E-12
5D_ProTAME stabilizes CyclinA2	proAAME	40	0.06	0.08	Nocodazole	37	0.28	0.28	4.71E-14
					proTAME	30	0.95	0.99	1.12E-12
	Nocodazole	37	0.28	0.28	proTAME	30	0.95	0.99	7.38E-06

Figure	Condition 1	N1	Median (μm)	Average (μm)	Condition 2	N2	Median (μm)	Average (μm)	MW-Wilcoxon Statistical test p value
5E_ProTAME does not alter inter-kinetochore distance	DMSO	55	1.22	1.23	proTAME	55	1.28	1.26	0.23

Table S1. Statistical analysis (continued).

Figure	Condition 1	N1	Median (min)	Average (min)	Condition 2	N2	Median (min)	Average (min)	MW-Wilcoxon Statistical test p value
6A_ProTAME arrest is MAD2-dependent	Control siRNA DMSO	150	108	121.7	MAD2 siRNA DMSO	150	12	13.2	0
					Control siRNA Nocodazole	150	1812	1680.1	0
					Control siRNA proTAME	150	1488	1512.4	0
	MAD2 siRNA DMSO	150	12	13.2	MAD2 siRNA Nocodazole	150	36	98.2	0
					MAD2 siRNA proTAME	150	84	121.7	0
6C_ProTAME arrest is hesperadin-sensitive	DMSO	150	102	110.9	DMSO Hesperadin	150	96	98.7	0.05
	Taxol	150	2070	1946.9	Taxol Hesperadin	150	156	168.7	0
	proTAME	150	1272	1329.3	proTAME Hesperadin	151	228	262.2	0
	Nocodazole	150	2094	1851.8	Nocodazole Hesperadin	150	552	579.4	0
6D_UbcH10 and Cdc27 siRNA induced mitotic delays are hesperadin-sensitive	Control siRNA	150	96	134.5	Control siRNA Hesperadin	150	120	115.6	0.22
					UbcH10 siRNA	150	156	330.5	1.51E-10
					Cdc27 siRNA	150	276	575.6	0
	UbcH10 siRNA	150	156	330.5	UbcH10 siRNA Hesperadin	150	108	115.8	5.20E-10
	Cdc27 siRNA	150	276	575.6	Cdc27 siRNA Hesperadin	150	132	131.8	0
7A_MG132 arrest is MAD2-dependent	Control siRNA 3µM MG132	169	840	830.1	MAD2 siRNA 3µM MG132	150	667.5	605.3	3.81E-05
7B_Hesperadin overrides MG132 3µM arrest in HeLa cells	MG132 3µM Hesperadin	150	174	502.2	MG132 3µM DMSO	154	1440	1549.9	0
					MG132 3µM Hesperadin proTAME	150	1296	1334.0	0
7C_Taxol does not restore mitotic arrest in the presence of MG132 3µM and hesperadin	MG132 3µM Hesperadin Taxol	151	420	705.3	MG132 3µM Hesperadin	155	312	511.4	0
					MG132 3µM Hesperadin proTAME	150	1524	1598.9	0
7D_Hesperadin overrides MG132 10µM arrest in HeLa cells	MG132 10µM Hesperadin	149	2160	2185.3	MG132 10µM DMSO	150	2172	2169.8	0.95
					MG132 10µM Hesperadin proTAME	119	2724	2657.5	1.35E-14

Table S1. Statistical analysis (continued).

Figure	Condition 1	N1	Median (min)	Average (min)	Condition 2	N2	Median (min)	Average (min)	MW-Wilcoxon Statistical test p value
8A_Cycloheximide sensitivity of mitotic arrests.	Nocodazole	70	2160	1777.0	Nocodazole Cycloheximide	70	444	442.5	0
	Taxol	100	1602	1557.6	Taxol Cycloheximide	100	516	538.6	0
	proTAME	100	972	1052.0	proTAME Cycloheximide	100	1608	1577.9	0
8B_MG132 arrest is MAD2-dependent (see cell fate distribution in figure)	Control siRNA MG132 10µM	66	1614	1715.1	Control siRNA MG132 10µM Cycloheximide	81	1788	1897.2	0.03
	MAD2 siRNA MG132 10µM	64	1716	1755.2	MAD2 siRNA MG132 10µM Cycloheximide	54	834	1078.0	3.11E-05
8C_Hesperadin rapidly overrides 10µM MG132 arrest in the presence of cycloheximide	MG132 10µM Cycloheximide Hesperadin	133	1104	1335.9	MG132 10µM DMSO Cycloheximide	151	2520	2346.4	0
					MG132 10µM Cycloheximide Hesperadin proTAME	132	2532	2383.9	0
S4A_ProTAME treatment arrests hTERT-RPE1 cells in mitosis.	DMSO	26	24	30.9	proAAME 12µM	31	36	32.1	0.25
					proTAME 6µM	24	522	819.0	8.15E-10
S4C_ProTAME induces a mild delay in chromosome congression	Untreated	96	21	21.5	proTAME 3µM	93	21	21.7	0.66
					proTAME 12µM	95	24	26.0	1.37E-09
					MG132 10µM	52	21	21.6	0.86
					Nocodazole 10nM	91	24	26.2	7.76E-08
S5G_Hesperadin overrides 12 µM proTAME-induced mitotic arrest after washout from a nocodazole-induced mitotic arrest	Release in medium	150	96	124.4	Release in proTAME 12µM	144	1410	1516.4	0
					Release in proTAME 12µM Hesperadin 100nM	150	156	202.1	1.05E-09
	Release in proTAME 12µM	144	1410	1516.4	Release in proTAME 12µM Hesperadin 100nM	150	156	202.1	0